Journal of Medical Virology 53:348–353 (1997)

Interspousal Transmission of GB Virus-C/Hepatitis G Virus: A Comparison With Hepatitis C Virus

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Although infection with GB virus-C/hepatitis G virus (GBV-C/HGV) by blood transfusion is well documented, little is known about the other routes of transmission. The prevalence of GBV-C/HGV infection in spouses of index patients and the related risk factors were studied. Hepatitis C virus (HCV) and GBV-C/HGV infections were studied in spouses of 100 patients with hepatitis C, of whom 12 were found to be also positive for GBV-C/HGV RNA. For couples both with GBV-C/HGV viremia, nucleotide sequences of the divergent envelope region were analyzed by phylogenetic tree constructions. For HCV infection, anti-HCV was found in 14 (14%) of the 100 spouses. Five spouses (42%) of the 12 patients with dual infection of GBV-C/HGV and HCV had evidence of GBV-C/HGV infection, three had viral RNA, and two had antibodies to a recombinant HGV envelope protein E2. Nucleotide sequence comparison and phylogenetic tree analysis of the genome in the GBV-C/HGV infected couple revealed the isolates to be closely related. These results suggest that spouses of patients with GBV-C/HGV infection are at a higher risk of acquiring GBV-C/HGV as compared with HCV, and they should be educated to avoid GBV-C/HGV infection from their spouses, in case GBV-C/HGV is shown to be pathogenic. ***J. Med. Virol. 53:348–353, 1997.***

KEY WORDS: anti-HGV-E2; phylogenetic tree analysis; spousal transmission

INTRODUCTION

Recently, two flavi-like RNA viruses were independently discovered from patients with chronic hepatitis and were designated GB virus-C (GBV-C) and hepatitis G virus (HGV), respectively [Simons et al., 1995; Leary et al., 1996; Linnen et al., 1996]. Both viruses are similar to hepatitis C virus (HCV), the major cause of parenteral non-A, non-B hepatitis worldwide, based on genomic organization and phylogenetic analysis [Simons et al., 1995; Leary et al., 1996; Linnen et al., 1996]. A further comparison of their putative encoded polyproteins shows a high sequence identity, indicating that GBV-C and HGV are actually different isolates of the same virus [Zuckerman 1996]. Thus "GBV-C/HGV" has been proposed to name this novel virus at present [Zuckerman 1996; Kao et al., 1997b]. Several studies using reverse transcription–polymerase chain reaction (RT-PCR) to detect GBV-C/HGV viremia in different populations showed that the virus is a transfusion-transmissible agent which can cause persistent infection in humans [Simons et al., 1995; Dawson et al., 1996; Kao et al., 1996b, 1997b; Linnen et al., 1996]. In addition, 10–25% of patients with chronic hepatitis C have been found to be also infected by GBV-C/HGV [Dawson et al., 1996; Linnen et al., 1996; Kao et al., 1997b], suggesting that both viruses may share similar modes of transmission.

Little is known about the transmission modes of GBV-C/HGV other than parenteral exposures. In hepatitis C, although not very efficient, sexual contact has been implicated in transmission of HCV [Alter 1994]. Several studies have also indicated that interspousal transmission is important for intrafamilial spread of HCV, with longer duration of marriage as the most evident risk factor [Kao et al., 1992, 1996c; Akahane et al., 1994; Chayama et al., 1995; Diago et al., 1996]. Whether this also occurs in the transmission of the novel GBV-C/HGV remains virtually unknown. Epidemiologic studies of the GBV-C/HGV have been ham-

Contract grant sponsor: Department of Health, ROC; Contract grant sponsor: National Science Council, Executive Yuan, ROC.

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Accepted 18 August 1997

pered by the lack of effective serologic markers of infection, and previous studies that have relied only on detection of viremia may lead to an underestimation of prevalence. Recently, an enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to a putative HGV envelope protein (anti-HGV-E2) has been developed, and preliminary results show that E2-specific antibodies are associated with loss of detectable GBV-C/HGV viremia [Dille et al., 1997; Tacke et al., 1997]. Thus, anti-HGV-E2 may be useful for the study of GBV-C/HGV exposure. In this communication, we reported the prevalence of GBV-C/HGV infection in spouses of index patients with dual infection of HCV and GBV-C/HGV by detecting GBV-C/HGV RNA as well as anti-HGV-E2 and investigated the possible risk factors. Nucleotide sequences from the divergent putative envelope region 2 (E2) of GBV-C/HGV genome of index cases and their spouses were further determined and analyzed by phylogenetic tree construction to provide molecular evidence of the interspousal transmission.

MATERIALS AND METHODS

Subjects

Spouses of 100 patients with chronic HCV infection (68 men and 32 women, age range: 28–76 years) followed at our hospital from September 1994 to December 1995 were studied. They were from an ongoing prospective study of interspousal HCV infection. Chronic HCV infection was defined by a positive reaction for second-generation antibodies against HCV (anti-HCV) (Abbott Laboratories, North Chicago, IL) for at least 6 months. Before blood sampling the couples were asked to complete a questionnaire addressing the occurrence of hepatitis, the duration of marriage, history and timing of any transfusion, injection drug usage (IDU) or nondisposable needle exposure, sexual behaviors, and sharing of personal tools such as toothbrush or razor. All of the index cases were anti-HCV–positive for >6 months at the time of blood sampling and were without concurrent hepatitis B virus infection, alcoholism, or markers suggestive of autoimmune diseases including antinuclear antibodies, antimitochondrial antibodies, and anti-smooth muscle antibodies. Among these, 75 had chronic hepatitis, 16 had hepatic cirrhosis, four had hepatocellular carcinoma, and five had normal liver tests. Possible sources of HCV infection were identified in 58 patients, i.e., blood transfusion in 40, surgery in 53 (including 19 with transfusion), and IDU in two. Accordingly, the exposure duration to their HCV-infected spouses could be estimated in these 58 couples. The duration of exposure was defined as the period from the time of HCV exposure to the present study if the event occurred after marriage, and it equaled the marriage duration if the exposure was premarital. The serum samples taken from each subject were stored at −70°C until use.

Serologic Testings

Serum samples were assayed for anti-HCV (Abbott Laboratories) and alanine aminotransferase (ALT). Anti-HGV-E2 was tested by a commercially available ELISA (Boehringer Mannheim, Germany).

Detection of Viral Genomes

Serum HCV RNA. Serum HCV RNA was assayed by RT-PCR with primers from the most conserved 5′ untranslated region (5′-UTR) of the viral genome [Kao et al., 1992].

Serum GBV-C/HGV RNA. Serum samples from all couples were subjected to the detection of GBV-C/HGV RNA by RT-PCR with nested primers derived from the highly conserved 5′-UTR of GBV-C/HGV genome [Kao et al., 1997a].

Amplification and sequencing of the putative E2 region of GBV-C/HGV genome. For couples with GBV-C/HGV viremia, the putative E2 region of the viral genome was amplified by using nested primers (outer sense: 5′-TCTGGAATACCTCTGGAAGG-3′, antisense: 5′-GCCTCCACCAGTGGTCGCGA-3′; inner sense: 5′-TGAGCAACGGATTGTCATGG-3′, antisense: 5′-GCGTGTGGGGTTCCGCTTCT-3′). This region was chosen because it is more divergent among different GBV-C/HGV isolates [Kao et al., 1997a]. The amplified DNA was directly sequenced by using fluorescence labeled primers with a 373A Sequencer (Applied Biosystems, Foster City, CA). The inner primer pairs were used as sequencing primers for both directions of the PCR products.

To avoid false-positive results, serum samples from healthy persons and reagents without DNA were used regularly as negative controls, and instructions to prevent cross-contaminations were strictly followed. Results were considered valid only when they were obtained consistently in at least two separate runs.

Phylogenetic analysis. A phylogenetic tree was constructed by using the program of Neighbor-Joining method (PHYLIP [Phylogeny Inference Package], version 3.5c; J. Felstein, University of Washington, Seattle) based on the nucleotide sequences of the amplified putative E2 region of the GBV-C/HGV genome.

Statistical Analysis

Data were analyzed by chi-square test with Yates' correction or by Fisher's exact test, if the expected number was less than 5, and unpaired *t*-test when appropriate. A P value of $<.05$ was considered significant.

RESULTS

Of 100 index patients with chronic HCV infection, 76 (76%) were positive for serum HCV RNA. Of the 100 spouses, anti-HCV was detectable in 14 (14%), and nine (64%) of them were positive for serum HCV RNA.

There was no statistical difference in sex distribution, mean age, mean peak serum ALT level, and risk factors of acquiring HCV infection including blood transfusion, surgery, and IDU between index patients with anti-HCV–positive and anti-HCV–negative spouses (Table I). Although the difference was not statistically significant, the mean marriage and exposure duration of index patients with anti-HCV–positive

TABLE I. Demographic and Clinical Characteristics of Chronic Hepatitis C Patients With and Without Seropositive Spouses*

Characteristics	Anti-HCV in spouses		*P* value
	Positive	Negative	
Number of cases	14	86	—
Male/female	10/4	58/28	NS
Age (years, mean ± SD)	53.1 ± 12.8	51.5 ± 11.4	NS
Liver disease (N/CH/LC/HCC)	1/11/1/1	4/64/15/3	—
Peak serum ALT (IU/L, mean ± SD)	118 ± 81	210 ± 241	NS
Risk factors of HCV infection (%)	6 (43)	52 (60)	NS
Blood transfusion (%)	4 (29)	36 (42)	NS
Surgery (%)	5 (36)	48 (56)	NS
Injection drug usage (%)	1 (7)	1 (1.6)	NS
Marriage duration (years, mean ± SD)	29.4 ± 13.8	25.1 ± 13.1	NS
Exposure duration[a] (years, mean ± SD)	18.7 ± 11.5	15.2 ± 10.9	NS
Sexual activity (times/week, mean ± SD)	2.0 ± 0.8	1.4 ± 0.6	.001
Condom usage (%)	2 (12)	11 (13)	NS
Toothbrush sharing (%)	2 (14)	1 (1.2)	.06

*NS, non-significant; N, normal; CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase.
[a]Estimated in 58 patients (six with seropositive spouses and 52 with seronegative spouses) from the time of previous blood transfusion, surgery, or injection drug usage.

TABLE II. Demographic and Clinical Characteristics of Chronic Hepatitis C Patients With and Without GBV-C/HGV Co-infection*

Characteristics	GBV-C/HGV RNA		*P* value
	Positive	Negative	
Number of cases	12	88	—
Male/female	6/6	62/26	NS
Age (years, mean ± SD)	47.1 ± 11.4	52.4 ± 11.5	NS
Liver disease (N/CH/LC/HCC)	0/9/2/1	5/66/14/3	—
Peak serum ALT (IU/L, mean ± SD)	207 ± 177	160 ± 151	NS
Risk factors of GBV-C/HGV infection (%)	12 (100)	49 (56)	.008
Blood transfusion (%)	7 (58)	33 (38)	NS
Surgery (%)	10 (83)	43 (49)	.05
Injection drug usage (%)	1 (8)	1 (1)	NS

*NS, non-significant; N, normal; CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase.

spouses was longer than that of patients with seronegative spouses. Of 60 spouses who had been married for longer than 20 years, anti-HCV was detected in 12 (20%) compared to 2 (5%) of 33 married for less than 20 years ($P = .03$). Consistent with previous results, there was more frequent sexual activity and more likelihood of sharing toothbrush in those with HCV-infected spouses (Table I). However, no significant difference was noted in the percentage of couples using condoms between the two groups.

By using RT-PCR assay, GBV-C/HGV genome was detectable in 12 of the 100 HCV-infected index cases (Table II), and those with dual infection of HCV and GBV-C/HGV had a higher frequency of having risk factors including blood transfusion, surgery, or IDU than those with HCV infection alone (100% vs. 56%, $P = .008$).

Among the spouses of the 12 index patients with GBV-C/HGV viremia, none was infected with HCV, while five spouses (42%) had evidence of GBV-C/HGV infection with GBV-C/HGV RNA detected in three and anti-HGV-E2 in two. There was no statistical difference in sex distribution, mean age, mean peak serum ALT level, risk factors of acquiring GBV-C/HGV infection, mean exposure duration, sexual activities, condom usage, and toothbrush sharing between spouses of index patients with and without GBV-C/HGV infection (Table III). All of the five GBV-C/HGV–infected spouses denied a history of blood transfusion, IDU or nondisposable needle exposure, premarital hepatitis, or sexual contacts with partners other than the index patients with dual infection. Although the difference was not statistically significant, the mean marriage duration of index patients with GBV-C/HGV–infected spouses was longer than that of patients with seronegative spouses. Of four spouses who had been married for longer than 30 years, GBV-C/HGV RNA was detected in two (50%) compared to one (13%) of eight married for less than 30 years.

Nucleotide sequences of the E2 region of the GBV-C/HGV genome could be directly sequenced and compared between each index case and the spouse in two of the three couples with GBV-C/HGV viremia; one couple had a 99% homology, and the other had a homology of 94% (Fig. 1). The sequences of the isolates between index patients and their spouses were compared by using phylogenetic analysis (Fig. 2). Based on the phylogenetic tree construction, each couple indeed had closely related isolates as compared with nonimpli-

TABLE III. Demographic and Clinical Characteristics of GBV-C/HGV-Infected Patients With and Without Seropositive Spouses*

Characteristics	GBV-C/HGV infection in spouses[a]		*P* value
	Positive	Negative	
Number of cases	5	7	—
Male/female	4/1	2/5	NS
Age (years, mean ± SD)	51.0 ± 9.2	45.8 ± 12.2	NS
Liver disease (N/CH/LC/HCC)	0/3/2/0	0/6/0/1	—
Peak serum ALT (IU/L, mean ± SD)	213 ± 70	205 ± 201	NS
Risk factors of GBV-C/HGV infection (%)	4 (80)	7 (100)	NS
Blood transfusion (%)	2 (40)	5 (71)	NS
Surgery (%)	2 (40)	7 (100)	NS
Injection drug usage (%)	0	1 (14)	NS
Marriage duration (years, mean ± SD)	25.6 ± 11.0	20.8 ± 12.1	NS
Exposure duration[b] (years, mean ± SD)	16.5 ± 4.9	15.2 ± 10.4	NS
Sexual activity (times/week, mean ± SD)	1.5 ± 0.5	1.8 ± 0.9	NS
Condom usage (%)	0	2 (29)	NS
Toothbrush sharing (%)	0	2 (29)	NS

*NS, non-significant; N, normal; CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase.
[a]GBV-C/HGV infection is the sum of GBV-C/HGV RNA positive values and anti-HGV-E2 positive values.
[b]Estimated in 11 patients (four with seropositive spouses and seven with seronegative spouses) from the time of previous blood transfusion, surgery, or injection drug usage.

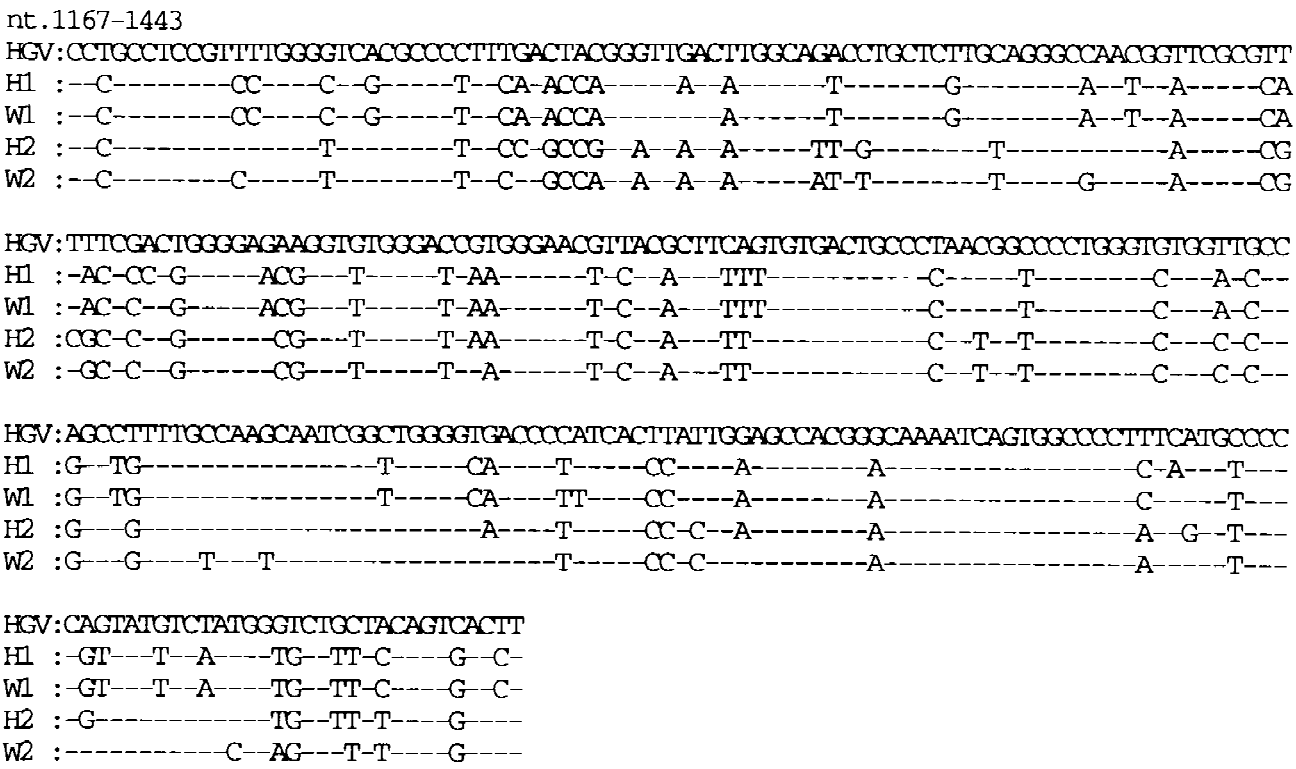

Fig. 1. Comparison of nucleotide sequences (nucleotide positions 1167–1443) of the putative envelope region 2 (E2) of GBV-C/HGV genome between two couples of spouses both with GBV-C/HGV viremia. The homology in couples 1 and 2 was 99% and 94%, respectively. The first row indicates the sequences from the prototype HGV strain [Linnen et al., 1996], and a dash denotes an identical nucleotide to the prototype sequence. H, husband; W, wife.

cated ones, consistent with the existence of interspousal transmission of GBV-C/HGV.

DISCUSSION

With the discovery of viral genome and subsequent development of molecular diagnostic assays [Simons et al., 1995; Leary et al., 1996; Linnen et al., 1996], the epidemiology and clinical significance of GBV-C/HGV infection are understood in part [Yoshiba et al., 1995; Dawson et al., 1996; Fiordalisi et al., 1996; Kao et al., 1996a, 1997b; Masuko et al., 1996; Wang et al., 1996]. It has been shown that GBV-C/HGV can be transmitted parenterally, and the infection seems not to cause significant hepatic damage as classic hepatitis viruses

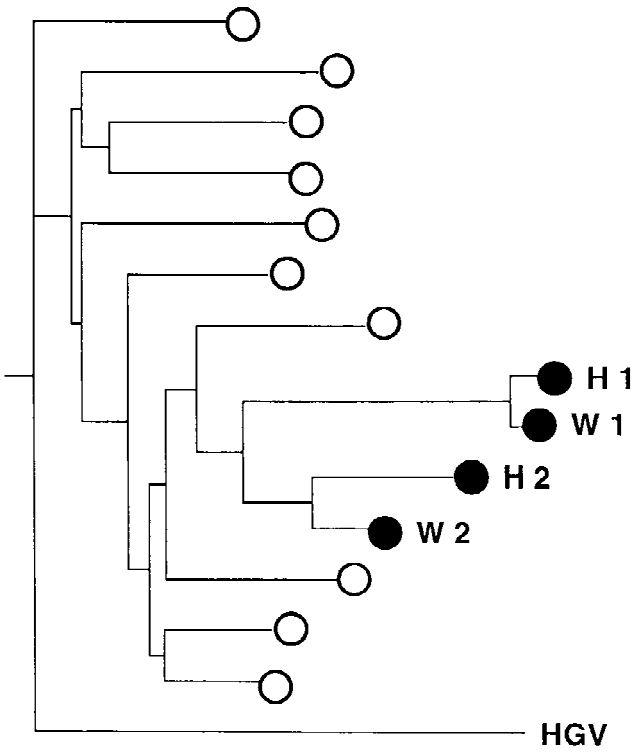

Fig. 2. Phylogenetic analysis of GBV-C/HGV isolates from two couples of spouses both with GBV-C/HGV viremia (H1 and W1, H2 and W2) based on the nucleotide sequences of the putative envelope region 2 of GBV-C/HGV genome shown in Figure 1. The phylogenetic tree was constructed by the Neighbor-Joining program in the PHYLIP package (version 3.5c). The open circles represent nonimplicated GBV-C/HGV isolates from other sources cloned in our laboratory. This tree is not rooted. H, husband; W, wife.

A–E do [Linnen et al., 1996; Masuko et al., 1996; Wang et al., 1996; Kao et al., 1997b]. Although transmission through blood transfusion or parenteral exposure is well documented for GBV-C/HGV, little is known about the possibilities of nonparenteral routes of its transmission. In HCV infection, previous studies have indicated that interspousal transmission of HCV exists, and a longer duration of marriage is one of the evident risk factors [Kao et al., 1992, 1996c; Akahane et al., 1994; Chayama et al., 1995; Diago et al., 1996]. However, whether this route also contributes to the spread of GBV-C/HGV, an HCV-like flavivirus, remains virtually unknown.

In the present study, the prevalence of anti-HCV in 100 spouses married to anti-HCV–positive index patients was 14%. In addition, index patients with anti-HCV–positive spouses had longer mean marriage and exposure durations and more frequent sexual activities than those without (Table I). These findings were in keeping with previous observations [Kao et al., 1992, 1996c], and thus transmission of HCV from index patients to their spouses over decades of close contact was indeed consistently found in Taiwan.

Co-infection of GBV-C/HGV has been observed frequently in patients with chronic hepatitis C [Dawson et al., 1996; Linnen et al., 1996; Kao et al., 1997b], and we have also noted that 12% of the HCV-infected index patients were positive for GBV-C/HGV RNA. This suggests that both viruses share common modes of transmission. Further studies on the clinical implications and interactions between HCV and GBV-C/HGV, which are flavi-like viruses, in dually infected patients are warranted.

The role of sexual contact in transmitting GBV-C/HGV has been unknown before. In this study, advantage was taken of high co-infection rate of GBV-C/HGV in patients with HCV infection to study the total prevalence of GBV-C/HGV infection in spouses of index patients who had a dual infection of HCV and GBV-C/HGV by the detection of viral RNA and anti-HGV-E2. Although the cases we studied were limited in number, five of 12 (42%) spouses married to patients with dual infection of HCV and GBV-C/HGV had evidence of GBV-C/HGV infection, and they were negative for anti-HCV as well as HCV RNA. In addition, nucleotide sequence comparison of the divergent putative E2 gene of GBV-C/HGV genome between index cases and their spouses in two couples revealed a high homology of 99% and 94%, respectively, and phylogenetic tree analysis documented that each couple virtually had the same isolate as compared to nonimplicated cases with GBV-C/HGV infection (Fig. 2). These data suggested that interspousal transmission of GBV-C/HGV indeed existed as has been found with HCV, and the efficiency of GBV-C/HGV transmission through this route seemed to exceed that of HCV (42% vs. 14%, $P = .9$). Further analysis of the demographic features, possible source of infection, and other risk factors showed comparable results between spouses of index patients with and without GBV-C/HGV infection (Table III). Nevertheless, the mean marriage duration of the index patients with seropositive spouses appeared to be slightly longer than that of patients with seronegative ones, although the difference did not reach a statistical significance (25.6 years vs. 20.8 years, $P > .5$). Couples married for >30 years had a higher frequency of GBV-C/HGV viremia than those married for <30 years (50% vs. 13%). These results implied that repeated exposures including sexual and/or nonsexual intimate contacts between spouses over time may be important in the transmission of GBV-C/HGV. Although parenteral exposures in the infected spouses were scrutinized carefully, the possibility that the couples were infected through a common external source cannot be excluded, and this is hard to prove based on the results of our cross-sectional study. Thus, a prospective study including more spouses of patients with GBV-C/HGV infection alone may provide further convincing evidence on this important issue.

In summary, spouses of GBV-V/HGV-infected patients are at increased risk of acquiring GBV-C/HGV infection with a transmission efficiency apparently higher than HCV, and the risk may increase over decades of exposure to infected partners. Whether those married to GBV-C/HGV RNA–positive subjects should be followed regularly for GBV-C/HGV markers and

whether preventive efforts to reduce the horizontal infection of GBV-C/HGV should be undertaken remain unsettled, mainly because the definite pathogenic roles of GBV-C/HGV have yet to be established [Alter et al., 1997a,b].

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